Effect of cyclooxygenase inhibitors on gentamicin-induced nephrotoxicity in rats

E.M. Hosaka[1],
O.F.P. Santos[3],
A.C. Seguro[2] and
M.F.F. Vattimo[1]

[1]Laboratório Experimental, Escola de Enfermagem, and
[2]Laboratório de Investigação Médica, Faculdade de Medicina,
Universidade de São Paulo, São Paulo, SP, Brasil
[3]Divisão de Nefrologia, Departamento de Clínica Médica,
Universidade Federal de São Paulo, São Paulo, SP, Brasil

Abstract

Correspondence

M.F.F. Vattimo

Escola de Enfermagem, USP

Av. Dr. Enéas C. Aguiar, 419

05403-000 São Paulo, SP

Brasil

Fax: +55-11-3066-7546

E-mail: nephron@usp.br

The frequent use of nonsteroidal anti-inflammatory drugs (NSAID) in combination with gentamicin poses the additional risk of nephrotoxic renal failure. Cyclooxygenase-1 (COX-1) is the main enzyme responsible for the synthesis of renal vasodilator prostaglandins, while COX-2 participates predominantly in the inflammatory process. Both are inhibited by non-selective NSAID such as indomethacin. Selective COX-2 inhibitors such as rofecoxib seem to have fewer renal side effects than non-selective inhibitors. The objective of the present study was to determine whether the combined use of rofecoxib and gentamicin can prevent the increased renal injury caused by gentamicin and indomethacin. Male Wistar rats (250-300 g) were treated with gentamicin (100 mg/kg body weight, ip, N = 7), indomethacin (5 mg/kg, orally, N = 7), rofecoxib (1.4 mg/kg, orally, N = 7), gentamicin + rofecoxib (100 and 1.4 mg/kg, respectively) or gentamicin + indomethacin (100 and 5 mg/kg, respectively, N = 8) for 5 days. Creatinine clearance and α-glutathione-S-transferase concentrations were used as markers of renal injury. Animals were anesthetized with ether and sacrificed for blood collection. The use of gentamicin plus indomethacin led to worsened renal function (0.199 ± 0.019 ml/min), as opposed to the absence of a nephrotoxic effect of rofecoxib when gentamicin plus rofexicob was used (0.242 ± 0.011 ml/min). These results indicate that COX-2-selective inhibitors can be used as an alternative treatment to conventional NSAID, especially in situations in which risk factors for nephrotoxicity are present.

Introduction

The interaction between gentamicin and other nephrotoxic drugs such as nonsteroidal anti-inflammatory drugs (NSAID) has not been emphasized despite its extreme clinical relevance. Gentamicin is often prescribed to patients with infections and with other symptomatic co-morbidities such as pain and inflammation, situations that require the application of anti-inflammatory drugs.

Treatment with gentamicin and NSAID is known to result in nephrotoxicity and the exact mechanism is still not completely un-

derstood. Some studies have provided evidence of an increased synthesis of renal vasodilatory prostaglandins in animals treated with gentamicin (1), an effect probably representing a compensatory mechanism for the prevention of a reduction in glomerular filtration rate during the development of gentamicin-induced nephrotoxicity. NSAID nonspecifically inhibit cyclooxygenase (COX), an enzyme involved in renal prostaglandin synthesis, and this inhibition is believed to promote compensatory vasodilatory disequilibrium, leading to a deterioration of glomerular filtration rate (1,2).

The discovery in 1991 of the existence of two COX isoforms (COX-1 and COX-2) and of their distinct physiological roles (3,4) permitted the development of COX-2-selective NSAID inhibitors, potentially revolutionary drugs able to alleviate COX-2-induced symptoms without the gastric and renal side effects caused by the inhibition of COX-1.

The aim of the present study was to determine whether the combined use of the COX-2-selective inhibitor rofecoxib and gentamicin can prevent the potentiation of renal injury caused by gentamicin when given with indomethacin. For this purpose, two groups of rats were studied: one receiving gentamicin and indomethacin and the other receiving gentamicin and rofecoxib.

Material and Methods

Male Wistar rats weighing 250-300 g and receiving water and ration *ad libitum* were randomly divided into six groups submitted to 5 days of treatment with the following drugs: saline group (N = 8), 0.1 ml 0.9% NaCl intraperitoneally (*ip*); gentamicin group (N = 7), gentamicin (Schering-Plough Laboratory, São Paulo, SP, Brazil) 100 mg/kg body weight, *ip*; rofecoxib group (N = 7), rofecoxib (Merck Sharp & Dohme Laboratory, São Paulo, SP, Brazil) 1.4 mg/kg body weight, orally; indomethacin group (N = 7), indomethacin (Merck Sharp & Dohme Labo-

ratory) 5 mg/kg body weight, orally; gentamicin + rofecoxib group (N = 7), gentamicin plus rofecoxib at doses of 100 and 1.4 mg/kg, respectively; gentamicin + indomethacin group (N = 8), gentamicin plus indomethacin at doses of 100 and 5 mg/kg, respectively.

The indomethacin and rofecoxib doses used in the present study were similar to the therapeutic doses used in patients.

Experimental design

After the last dose, all animals were placed in metabolic cages for 24 h, with water and ration available *ad libitum*. The animals were then anesthetized with ether for blood collection by puncture of the abdominal aorta and sacrificed according to the norms for didactic-scientific practice of animal vivisection. The kidneys were removed and placed in 35% formaldehyde for histological analysis.

Blood and urine samples were used for the determination of creatinine clearance by the modified Jaffe method.

A 400-µl urine sample was used to quantify urinary α-glutathione-S-transferase (α-GST), an enzyme marker for the identification of proximal tubular injury. After collection, a urine stabilizer (100 µl thiomersal and sodium azide per 400 µl urine) was added to the sample, which was kept at -20ºC for later determination. α-GST was quantified by ELISA using the Biotrin Rat Alpha-GST kit (Biotrin International, Dublin, Ireland).

Determination of creatinine clearance by the Jaffe method

Plasma was deproteinized for the determination of serum creatinine with a spectrophotometer at 520 nm absorbance. For urinary creatinine, absorbance was measured as described above. Creatinine clearance was calculated by multiplying urinary creatinine by 24-h urinary flow (in ml/min), divided by plasma creatinine.

Quantification of urinary α-glutathione-S-transferase by ELISA

The samples were diluted 1:5 in sample diluent (Biotrin Rat α-GST), i.e., 100 μl urine per 400 μl diluent. The urine samples were then added to uncovered plates containing rat anti-α-GST IgG antibody, on which the α-GST in the sample was captured by the anti-α-GST antibody attached to the plate. The following substances were then added, with the plates being washed between each new addition: enzymatic rat anti-α-GST IgG conjugate, enzyme-conjugated anti-IgG antibody, and an enzyme cholerigenic substrate. The intensity of the resulting color was proportional to the amount of α-GST present in the sample. Finally, absorbance was measured with an ELISA plate reader using a 450-nm filter.

Renal histology

For histological evaluation of the renal tubular structure by light microscopy, the kidneys were fixed in 35% formaldehyde, paraffin-embedded, sectioned, and prepared on slides stained with hematoxylin and eosin.

Statistical analysis

Statistical analyses were performed using the SPSS software, version 8.0 (SPSS Inc., Chicago, IL, USA, 1989-1997).

The Levene test used analysis of variance homogeneity between groups. Multiple comparisons between creatinine clearance/100 g (CrCl/100 g) body weight and α-GST, based on the mean values obtained for each group, were performed using the Student t-test and the Mann-Whitney test, with the level of significance set at P < 0.05.

Results

As shown in Table 1, gentamicin treatment led to a significant reduction in CrCl/

100 g, while urinary flow was constant, suggesting the occurrence of non-oliguric acute renal failure of nephrotoxic origin. The saline group was within the normal range reported in previous studies (5).

Treatment with the anti-inflammatory drugs rofecoxib and indomethacin did not lead to significant alterations in renal function, with creatinine clearance and urinary flow being similar to those observed for the control group.

Drug combination led to a significant decrease in CrCl/100 g in the gentamicin + rofecoxib group compared to the saline and rofecoxib groups, without alterations in urinary flow. The gentamicin + indomethacin combination caused a significant reduction in CrCl/100 g with maintenance of urinary flow compared to the saline, gentamicin and indomethacin groups. This result demonstrates that the combination of a nonspecific COX inhibitor (indomethacin) and gentamicin not only induces nephrotoxic acute renal failure but also potentiates the nephrotoxic effect of the aminoglycoside, as shown by the comparison with the gentamicin group. This was not observed for the gentamicin and rofecoxib combination.

No significant differences were observed between the gentamicin + indomethacin and gentamicin + rofecoxib groups. In addition,

Table 1. Effect of gentamicin, rofecoxib and indomethacin on renal function and creatinine clearance parameters.

Groups	U (ml/min)	CrU (mg/dl)	CrP (mg/dl)	CrCl/100 g
Saline	0.0074 ± 0.0009	100 ± 8	0.416 ± 0.037	0.679 ± 0.070
Gentamicin	0.0057 ± 0.0011	93 ± 16	0.601 ± 0.073	0.259 ± 0.018*
Rofecoxib	0.0062 ± 0.0008	91 ± 12	0.337 ± 0.018	0.580 ± 0.054
Indomethacin	0.0057 ± 0.0005	111 ± 9	0.387 ± 0.024	0.620 ± 0.098
Gentamicin + rofecoxib	0.0045 ± 0.0005	91 ± 7	0.579 ± 0.038	0.242 ± 0.011*+
Gentamicin + indomethacin	0.0052 ± 0.0009	70 ± 9	0.657 ± 0.060	0.199 ± 0.019*‡§

Data are reported as means ± SEM. U = urinary flow; CrU = urinary creatinine; CrP = plasma creatinine; CrCl = creatinine clearance.
*P < 0.05 *vs* saline; +P < 0.05 *vs* rofecoxib; ‡P < 0.05 *vs* indomethacin; §P < 0.05 *vs* gentamicin (Mann-Whitney and *t*-test).

significance was close to α (P = 0.054), with an unfavorable tendency towards renal toxicity being observed for the gentamicin + indomethacin group.

As shown in Table 2, the gentamicin group showed a statistically significant increase in urinary α-GST compared to the control group, indicating gentamicin-induced proximal tubular damage. Similar to the creatinine clearance results, the urinary α-GST concentrations for the rofecoxib and indomethacin groups were similar to those for the control group, indicating that neither NSAID caused proximal tubular renal damage.

A significant difference in α-GST was also observed when the NSAID were used in combination with gentamicin, with the gentamicin + rofecoxib and gentamicin + indomethacin groups showing higher urinary α-GST levels than the control group. This increase in urinary α-GST levels upon combined treatment with these two types of drugs indicates that gentamicin was responsible for the occurrence of renal injury, since neither NSAID alone caused any proximal tubular damage.

An increase in urinary α-GST excretion was observed for the gentamicin + rofecoxib group compared to the rofecoxib group. On the other hand, no significant difference in urinary α-GST was observed between the gentamicin + indomethacin and indomethacin groups. These results suggest a discrete worsening in gentamicin-induced nephro-

Table 2. Effect of gentamicin, rofecoxib and indomethacin on urinary α-GST.

Groups (N)	α-GST (μg/l)
Saline (7)	13.74 ± 0.42
Gentamicin (7)	23.10 ± 3.59*
Rofecoxib (8)	13.54 ± 0.29
Indomethacin (6)	16.23 ± 2.45
Gentamicin + rofecoxib (8)	20.96 ± 2.92*+
Gentamicin + indomethacin (8)	21.35 ± 2.34*

Data are reported as means ± SEM.
*P < 0.05 *vs* saline; +P < 0.05 *vs* rofecoxib (Mann-Whitney and *t*-test).

toxic renal failure when a nonspecific COX inhibitor (indomethacin) instead of a COX-2-selective inhibitor (rofecoxib) was used in combination.

Histology

Histological analysis of kidney sections showed varying morphological patterns ranging from the absence of histological alterations to minimal changes. It should be noted that the observed histological patterns did not correlate with type of treatment and therefore could not be used to define a histological profile for the kidneys of animals submitted to saline, gentamicin or the drug combinations studied. Thus, the histological analysis employed here was probably not sensitive enough to detect possible structural alterations caused by the different treatments or functional changes induced by drug treatment, at the time studied.

Discussion

The results of the present study suggest that the selective inhibition of COX-2 apparently does not cause further damage in addition to the nephrotoxic effect caused by gentamicin. As expected, all rats treated with gentamicin showed a reduction in renal function, including those receiving gentamicin in combination with NSAID.

Gentamicin-induced nephrotoxicity is a well-documented event involving some functional and cellular mechanisms such as glomerular lesions that interfere with glomerular hemodynamics, and altered tubular transport, with the injury ranging from solely functional lesions to the occurrence of necrosis (6).

In the present study, the administration of gentamicin caused a significant reduction in glomerular filtration rate, as identified by reduced creatinine clearance. Experimental studies have demonstrated that this aminoglycoside causes a significant reduction in

K_f and an increase in the resistance of afferent and efferent arterioles, temporarily associated with a 30 to 50% reduction in global renal and nephron filtration (7,8). This reduced filtration seems essentially to be related to an obstructive renal intratubular mechanism which reduces intraluminal flow, a defect affecting most functional renal tubules. This altered flow, in turn, may trigger the decline in final excretion (9). In addition to the obstructive component, glomerular filtrate leakage through the damaged tubular epithelium may also contribute to the decrease in glomerular filtration.

The decrease in creatinine clearance corresponds to the reduction in the glomerular filtration rate mentioned above, indicating that the hemodynamic mechanism established here reproduced the events described above, including a reduction in K_f, obstruction and tubular leakage.

In addition to causing changes in filtration, gentamicin has been shown to induce proximal tubular injury, with the damage ranging from alterations in tubular reabsorption to necrosis of proximal tubule cells. The mechanisms leading to this event remain unclear, but interactions of the drug with the cell membrane and intracytoplasmic organelles cannot be ruled out (10).

Therefore, enzymatic markers naturally excreted during organ damage have been used in addition to the determination of creatinine clearance. The best known markers are N-acetyl-beta-D-glucosaminidase (NAG), alanine-aminopeptidase and ß2-microglobulin (11). Comparative studies regarding the predictive value of these enzymes for the evaluation of gentamicin nephrotoxicity have revealed the efficacy and superiority of NAG over the other markers, a fact attributable to the proportional relationship between NAG and gentamicin-induced renal damage (11).

Due to its advantages in the identification of renal injury, α-GST was used in the present study as a complementary parameter for the evaluation of proximal tubular damage. The results showed an elevated concentration of this enzyme in the groups submitted to treatment with gentamicin alone or in combination with the anti-inflammatory drugs. These findings confirm the damaging effects of this aminoglycoside in the proximal tubular region, probably as a result of the cell mechanisms discussed at the beginning of this section.

Similar urinary α-GST levels were observed for the groups receiving only anti-inflammatory drugs and the control group, similar to the results obtained for creatinine clearance. Taken together, these findings suggest that the anti-inflammatory drugs used in the present study did not cause functional alterations or tubular damage in these animals, thus confirming previous studies on the toxicity of these drugs.

On the other hand, the decline in glomerular filtration rate may stimulate the synthesis of vasodilatory prostaglandins that act in the reduction of afferent arteriolar resistance and thus contribute to the reestablishment of renal plasma flow. The use of NSAID may inhibit this additional protective mechanism, since afferent arteriolar tonus is increased in the absence of dilatory prostaglandins, leading to a decline in renal plasma flow associated with a reduced K_f, a situation that results in a dramatic reduction in glomerular filtration rate (12).

Similar to gentamicin, other drugs also show preferential toxicity for the kidney, including the COX-inhibiting NSAID such as indomethacin. In addition to nephrotoxicity, indomethacin also presents gastrointestinal toxicity. Gastric toxicity is one of the most common side effects and manifests in the form of dyspepsia, gastritis and peptic ulcers (13).

All the gastric and renal effects of NSAID are directly related to their potential for inhibiting the COX-1 isoform, which is constitutively expressed in the kidney. The inhibition of COX-1 is in turn related to the inhibition of the synthesis of renal vasodilatory

prostaglandins such as PGE2 and PGI2, causing the above described effects.

In the present study, indomethacin treatment alone did not alter renal function as determined by creatinine clearance. Furthermore, no significant increase in urinary α-GST levels was observed, thus excluding any proximal tubular damage. This finding confirms previous studies which reported that the toxic effects of this drug are limited to situations of pre-existing damage, or when the drug is administered in combination with other nephrotoxic drugs.

As described previously (2,14) and confirmed in the present study, exclusive administration of gentamicin resulted in a marked reduction in glomerular filtration rate, as indicated by a significantly lower creatinine clearance compared to the control group. When gentamicin was administered in combination with indomethacin, a significantly higher decrease in creatinine clearance was observed compared to the group treated with gentamicin alone.

Evaluation of the nephrotoxicity of gentamicin administered in combination with an NSAID showed that the NSAID potentiated the reduction in urinary concentrating capacity observed with gentamicin alone after 10 days of treatment (1). In addition, animals treated with gentamicin alone showed a specific increase in PGE2, a renal vasodilator. These results suggest that the NSAID impairs the PGE2-mediated compensatory vasodilatory mechanism for the maintenance of glomerular filtration rate and renal blood flow, thus extending the renal damage caused by gentamicin (1).

The results of the present study suggest that, although the administration of indomethacin alone did not affect normal renal function, its combination with gentamicin potentiated renal damage, in view of the decline in glomerular filtration rate evidenced by the reduced creatinine clearance in the gentamicin group and intensified by the administration of indomethacin. Thus, indo-

methacin may have caused the inhibition of vasodilatory prostaglandins that physiologically act on the preservation of renal plasma flow in this model of acute renal failure. It should be emphasized here that COX-1 is known to promote renal vasodilatation, especially in the presence of other renal damage-causing factors (e.g., aminoglycosides).

Another mechanism whereby nonselective NSAID inhibitors of COX may facilitate vasoconstriction and extend ischemic lesions involves an increase in the formation of vasoconstrictive metabolites derived from arachidonic acid, since the inhibition of COX makes a larger amount of this acid available for metabolization through COX-independent routes, thus producing a series of potent vasoconstrictors (15,16).

Both COX-2 and COX-1, although constitutively expressed in some parts of the kidney (macula densa, interstitial medullary and epithelial cells adjacent to the thick cortical ascending segment), are considered to be induced during inflammatory events (17,18). However, little is known thus far about their action under conditions of renal deficiency.

In the present study, the exclusive use of a COX-2-selective inhibitor (rofecoxib) did not alter renal function. Therefore, the results obtained for the rofecoxib and indomethacin groups indicate that the inhibition of prostaglandin synthesis in healthy animals by both anti-inflammatory drugs does not affect normal renal function.

In contrast, the combination of gentamicin and the COX-2-selective NSAID led to a reduction in creatinine clearance similar to that caused by gentamicin alone and slightly less intense than that observed for the gentamicin + indomethacin group. This result suggests that the selective inhibition of COX-2 induced by the selective NSAID did not interfere with the gentamicin-induced mechanisms of nephrotoxicity nor did it exert other damaging effects on renal function.

Although no marked difference in renal

function was observed between animals treated with gentamicin and animals treated with the nonspecific or COX-2-selective NSAID, renal injury was more intense when the nonspecific NSAID was used in combination. A possible explanation for this result is the preservation of the synthesis of vasodilatory prostaglandins such as PGE2 by the COX-2-selective inhibitors, stimulated by gentamicin.

Acknowledgments

References

1. Assael BM, Chiabrando C, Gagliardi L, Noseda A, Bamonte F & Salmona M (1985). Prostaglandins and aminoglycoside nephrotoxicity. *Toxicology and Applied Pharmacology*, 78: 386-394.

2. Farag MM, Mikhail M, Shehata R, Abdel-Meguid E & Abdel-Tawab S (1996). Assessment of gentamicin-induced nephrotoxicity in rats treated with low doses of ibuprofen and diclofenac sodium. *Clinical Science*, 91: 187-191.

3. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB & Lipsky PE (1998). Cyclooxygenase in biology and disease. *FASEB Journal*, 12: 1063-1073.

4. Vane JR & Botting RM (1995). New insights into the mode of action anti-inflammatory drugs. *Inflammation Research*, 44: 1-10.

5. Dorea EL, Yu L, De Castro I, Campos SB, Ori M, Vaccari EM, Lacaz C da S & Seguro AC (1997). Nephrotoxicity of amphotericin B is attenuated by solubilizing with lipid emulsion. *Journal of the American Society of Nephrology*, 8: 1415-1422.

6. Humes HD (1988). Aminoglycoside nephrotoxicity. *Kidney International*, 33: 900-911.

7. Baylis C, Rennke HG & Brenner BM (1977). Mechanisms of the defect in glomerular ultrafiltration associated with gentamicin administration. *Kidney International*, 12: 344-353.

8. Schor N, Ichikawa I, Renke HG, Troy JL & Brenner BM (1981). Pathophysiology of altered glomerular function in aminoglycoside-treated rats. *Kidney International*, 19: 288-296.

9. Brady HR, Brenner BM, Clarkson MR & Lieberthal W (2000). Acute renal failure. In: Brenner BM (Editor), *The Kidney*. WB Saunders Company, Philadelphia, PA, USA.

10. Gibey R, Dupond JL, Alber D, Leconte des Floris R & Henry JC (1981). Predictive value of urinary N-acetyl-beta-D-glucosaminidase (NAG), alanine-aminopeptidase (AAP) and beta-2-microglobulin (beta 2M) in evaluating nephrotoxicity of gentamicin. *Clinica Chimica Acta*, 116: 25-34.

11. Mondorf AW, Breier J, Hendus J, Scherberich JE, Mackenrodt G, Shah PM, Stille W & Schoeppe W (1978). Effect of aminoglycosides on proximal tubular membranes of the human kidney. *European Journal of Clinical Pharmacology*, 13: 133-142.

12. Cronin RE & Henrich WL (2000). Toxic nephropathy. In: Brenner BM (Editor), *The Kidney*. WB Saunders Company, Philadelphia, PA, USA.

13. Polisson R (1996). Nonsteroidal anti-inflammatory drugs: practical and theoretical considerations in their selection. *American Journal of Medicine*, 100 (Suppl 3A): 31S-36S.

14. Higa EMS, Schor N, Boim MA, Ajzen H & Ramos OL (1981). Role of the prostaglandin and kallikrein-kinin systems in aminoglycoside-induced acute renal failure. *Brazilian Journal of Medical and Biological Research*, 18: 355-365.

15. Giovanni G & Giovanni P (2002). Do non-steroidal anti-inflammatory drugs and COX-2 selective inhibitors have different renal effects? *Journal of Nephrology*, 15: 480-489.

16. Schlondorff D (1993). Renal complications of nonsteroidal anti-inflammatory drugs. *Kidney International*, 44: 643-653.

17. Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN & Breyer MD (1994). Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *Journal of Clinical Investigation*, 94: 2504-2510.

18. Lipsky PE (1999). Role of cyclooxygenase-1 and 2 in health and disease. *American Journal of Orthopedics*, 28 (Suppl 3): 8-12.